

# Microelectrochemical sensors for *in vivo* brain analysis: an investigation of procedures for modifying Pt electrodes using Nafion®



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Received 9th January 2003, Accepted 26th February 2003  
First published as an Advance Article on the web 3rd April 2003

Various Nafion® coating procedures were examined in order to design a simple and reproducible coating method to maximise permselective characteristics, and thus eliminate signals from electroactive interferents, in sensors designed for direct *in vivo* measurements in the brain. Interferents investigated included ascorbic acid (AA), the principal endogenous electroactive interferent present in the brain, and uric acid. Application of the Nafion® (5% commercial solution) using a thermally annealing procedure involving 5 pre-coats, and 2 subsequent dip-bake layers resulted in elimination of interferent signals. It also produced complete blocking of the signal for the neurotransmitter dopamine. The optimum time and temperature for annealing was found to be 5 min at 210 °C. An examination of shelf life over two weeks indicated negligible AA interference over this period. Preliminary investigations with respect to the potential use of these Nafion®-modified Pt electrodes in the design of implantable, first generation, peroxide detecting biosensors indicated that the modified electrode had no effect on O<sub>2</sub> permeability but did produce a significant decrease in H<sub>2</sub>O<sub>2</sub> sensitivity. While this may preclude their use in biosensor development they may be more suitable for detection of gaseous neurochemicals such as nitric oxide.

## Introduction

Although ion-containing polymers have been around for some time the last ten years has seen a dramatic increase in the number of published research reports on these ionomers. The focus of most of this research effort has been devoted to only a small number of materials, notably the ethylenes, styrenes, rubbers, and those based on poly(tetrafluoroethylene).<sup>1</sup> Nafion® is one such example of a poly(tetrafluoroethylene) based ionomer.<sup>2</sup> It is a perfluorinated polymer that contains small proportions of sulfonic or carboxylic ionic functional groups. From its development by DuPont in the 1960s, it has been used in a broad range of applications including liquid and gas separations, fuel cells, and the chlor-alkali industries. This is primarily because of its thermal and chemical resistance, ion-exchange properties, high conductivity, selectivity, mechanical strength, and insolubility in water.

One novel application of Nafion® that has arisen in recent years is its use in the chemical modification of electrode surfaces. However, a survey of the literature in this field reveals a multitude of different polymer coating methods including, droplet evaporation or dip coating using the commercial 5% solution,<sup>3–8</sup> and various concentrations of alcohol<sup>3,9–15</sup> or water diluted<sup>16</sup> solutions; drying stationary<sup>3,4,7–9,11–13,15,16</sup> or spinning<sup>2</sup> at room temperature, using a hot air gun<sup>2</sup> or IR lamp;<sup>10–14</sup> baking at different temperatures (60 °C,<sup>17</sup> 80 °C,<sup>5</sup> 170 °C,<sup>18</sup> and 200 °C<sup>1</sup>); electrodeposition;<sup>6,17</sup> agitation in an ultrasonic bath;<sup>16</sup> and chemical modification into the sodium form of the polymer using NaOH.<sup>18</sup> As one would expect such diverse methods produce wide variations in the operational characteristics of the modified electrodes.

Nafion®-modified electrodes have previously been used for the detection of electroactive cationic neurotransmitter species in the ECF.<sup>19,20</sup> The coating shows low permeability to ascorbic acid (AA), the principal endogenous electroactive interferent and a monoanion at pH 7.4, and anionic neurotransmitter metabolites such as 3,4-dihydroxyphenylacetic acid and 5-hy-

droxyindoleacetic acid, while being highly selective for cationic species such as monoamines (*e.g.* dopamine).

More recently, Nafion® coatings have been used in the development of implantable biosensors designed for the detection of non-electroactive species in brain extracellular fluid (ECF).<sup>21–24</sup> However, in the design of these implantable biosensors the Nafion® is generally not applied on its own, but as part of a complex recipe usually involving various polymers, immobilising agents, and coating methodologies. Thus, in this paper we present results of a detailed systematic study of various Nafion® coating procedures performed in order to design a simple and reproducible coating method to maximise permselective characteristics and thus eliminate signals from electroactive interferents in sensors designed for direct *in vivo* measurements.

## Experimental

### Reagents and solutions

The Nafion® (1100 EW, 5 wt.% solution in a mixture of lower aliphatic alcohols and H<sub>2</sub>O) was obtained from Aldrich Chemical Co., Dorset, UK. The L-ascorbic acid (AA; A.C.S. reagent), uric acid (UA; sodium salt), dopamine (DA; hydrochloride) and H<sub>2</sub>O<sub>2</sub> (A.C.S. reagent, 30.4 %) were obtained from Sigma-Aldrich Ireland Ltd. The NaCl (SigmaUltra), NaH<sub>2</sub>PO<sub>4</sub> (Sigma, A.C.S. reagent) and NaOH (SigmaUltra) were used as supplied.

Stock standard solutions (100 mM) of all compounds were prepared at the beginning of each experiment to avoid problems associated gradual decomposition. The experiments were carried out in phosphate buffer saline (PBS) solution, pH 7.4 (0.15 M NaCl, 0.04 M NaH<sub>2</sub>PO<sub>4</sub> and 0.04 M NaOH), which was deaerated with O<sub>2</sub>-free N<sub>2</sub> for 20 min prior to commencing electrochemical measurements. All solutions were prepared using deoxygenated doubly distilled deionised water.

## Working electrode preparation

Nafion<sup>®</sup>-coated Pt disk electrodes were made from Teflon-insulated platinum/iridium (Pt/Ir 90%/10%) wire (125  $\mu\text{m}$  bare diameter, 160  $\mu\text{m}$  coated diameter (5T), Advent Research Materials, Suffolk, UK). The electrodes were approximately 5 cm in length and were prepared by carefully cutting 5 mm of the Teflon insulation from one end of the wire. A gold electrical contact (Semat Technical, Herts, UK) was soldered to this end of the wire to enable connection with the instrumentation. The other end of the wire acted as the active (disk) surface of the electrode. Various coating procedures for modifying the electrode surface with Nafion<sup>®</sup> were tested (see Nafion<sup>®</sup> application below). When not in use all electrodes were stored dry at room temperature.

### Nafion<sup>®</sup> application: dip method

All electrode surface coating of Nafion<sup>®</sup> was performed using the dip method. This involves dipping the active surface of the electrode into a fixed volume of the commercial 5% Nafion<sup>®</sup> solution. Briefly, a Nafion<sup>®</sup> droplet (5  $\mu\text{L}$ ) was placed onto a watch glass using a syringe. The electrode was then immediately dipped into, and removed from, the droplet. The Nafion<sup>®</sup> was let air dry for 2 min between successive coatings.

After application of the final coat the electrode was used as is or was then cured at either 85  $^{\circ}\text{C}$  or 210  $^{\circ}\text{C}$  for 5 min unless stated otherwise. This was achieved by placing the electrode on a watch glass in an oven (Model 19, 600 watts. Precision Scientific Group, Chicago, Illinois, USA) set at the appropriate temperature. An alternative to the latter single bake procedure was to dip-bake the electrode. This method involves the same procedures described above except that the electrode was cured at either 85  $^{\circ}\text{C}$  or 210  $^{\circ}\text{C}$  for 5 min after application of each individual Nafion<sup>®</sup> coat.

### Nafion<sup>®</sup> application: pre-cast method

The pre-cast method involves placing an under layer of concentrated Nafion<sup>®</sup> onto the electrode surface. This is achieved by placing a droplet of Nafion<sup>®</sup> onto a watch glass as before. This droplet is then allowed to air dry at room temperature for 5 min. After drying further individual drops (5 or 10, see below) are placed on top of the initial droplet using the same procedures. This produces a localised concentrated layer of Nafion<sup>®</sup> on the watch glass.

After either 5 or 10 drops (called 5 or 10 pre-coats) have been placed onto the watch glass as outlined, a further drop of Nafion<sup>®</sup> is then placed on top of this concentrated pre-coated Nafion<sup>®</sup> layer. The active surface of the electrode is dipped into this concentrated layer and, as before, the electrode is then immediately removed and let air dry at room temperature for 2 min. The final fresh Nafion<sup>®</sup> droplet is required to adhere the concentrated Nafion<sup>®</sup> layer to the electrode. This electrode is then placed into an oven and baked for 5 min at 210  $^{\circ}\text{C}$ . After baking, the electrode can be further coated with Nafion<sup>®</sup> layers by repeating the dip-bake procedure described above in Nafion<sup>®</sup> application: dip method.

### Characterisation of Pt/Nafion<sup>®</sup> electrodes

AA, UA, DA, O<sub>2</sub> and H<sub>2</sub>O<sub>2</sub> calibrations were performed in a standard three-electrode glass electrochemical cell which was constructed in house. A saturated calomel electrode (SCE) was used as the reference electrode and a large Pt wire served as the auxiliary electrode. To facilitate mixing, solutions were bubbled

with N<sub>2</sub> for *ca.* 5 s following the addition of each aliquot. The current was then measured when the solution was quiescent. The electrodes were held at a constant potential of +700 mV, which is the value generally used for implantable electrochemical biosensors.<sup>25</sup> All calibrations were performed at room temperature.

Oxygen calibrations were performed by recording the current in PBS saturated with either N<sub>2</sub> (British Oxygen Co. (BOC), Dublin, Ireland) or air (RENA 102 air pump), where the concentration of solution O<sub>2</sub> has been reported as 0 and 200  $\mu\text{M}$ .<sup>26,27</sup>

### Instrumentation and software

Constant potential amperometry was performed in all experiments using a low-noise potentiostat (Biostat II, Electrochemical and Medical systems, Newbury, UK). Data acquisition was carried out with a Gateway GP6-350 computer, a Powerlab/400 interface system (ADInstruments Ltd, East Sussex, UK) and Chart for Windows (v4.0.1) software (ADInstruments Ltd). All analysis was performed using Microsoft Excel. Data are represented as mean  $\pm$  SEM. The significance of differences observed was estimated using either the Student's *t*-test (two-tailed) for unpaired observations or the Mann-Whitney Test (InStat v3.0.5, GraphPad Software Inc., CA, USA).

## Results and discussion

Ascorbic acid (AA), the principal endogenous electroactive interferent present in the brain has a high baseline level (*ca.* 300–500  $\mu\text{M}$ ) and continuously changing extracellular concentration.<sup>28</sup> As such, sensitivity to AA (1 mM) was used in order to determine the suitability of the various Nafion<sup>®</sup>-coated electrodes for *in vivo* measurements.

### Number of Nafion<sup>®</sup> coats

We began our study by investigating the simplest approach of applying Nafion<sup>®</sup> onto the electrode surface; dip coating using the commercial 5% solution and allowing the polymer to dry (2 min) at room temperature between coats. Fig. 1 shows the steady-state current concentration profiles for 1, 2, 5, 6 and 10 coats. All the responses represented a significant decrease in AA sensitivity from that observed at bare Pt ( $45.22 \pm 1.57 \text{ nA mM}^{-1}$ ,  $n = 30$ ) and were linear (mean  $r^2 = 0.997 \pm 0.001$ ) with sensitivity decreasing with increased number from 1 up to 6:  $16.61 \pm 0.90 \text{ nA mM}^{-1}$  (1,  $n = 4$ ,  $P < 0.0001$ );  $7.45 \pm 0.62 \text{ nA mM}^{-1}$  (2,  $n = 6$ ,  $P < 0.0001$ );  $4.85 \pm 0.86 \text{ nA mM}^{-1}$  (5,  $n = 4$ ,  $P < 0.0001$ );  $0.75 \pm 0.23 \text{ nA mM}^{-1}$  (6,  $n = 5$ ,  $P < 0.0001$ ).

Increasing the number of coats from 6 to 10 ( $0.52 \pm 0.06 \text{ nA mM}^{-1}$ ,  $n = 5$ ) resulted in no significant decrease in sensitivity ( $P = 0.4038$ , see inset Fig. 1), indicating that for optimum AA blocking at least six coatings are required when using the commercial 5% solution neat.

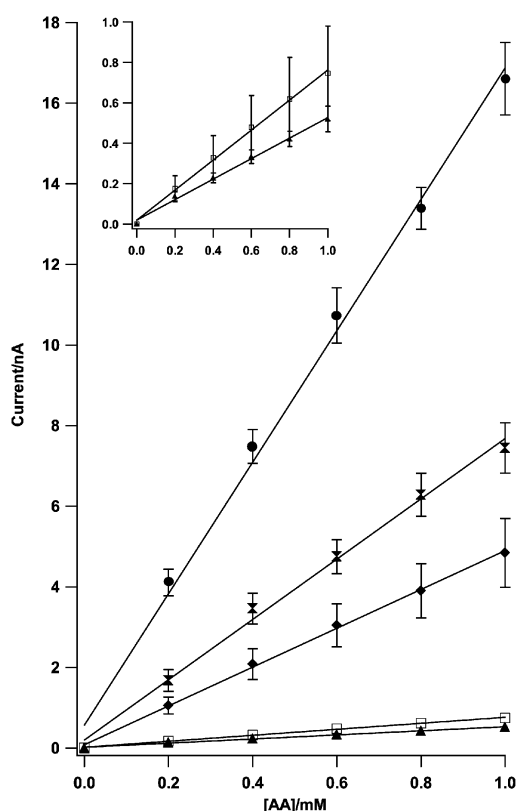
### Drying time

We next examined the effect of altering the room temperature drying time between the application of the ten Nafion<sup>®</sup> coats. Increasing the time from 2 min to 10 min resulted in a deterioration in the blocking ability with the AA sensitivity increasing from  $0.52 \pm 0.06 \text{ nA mM}^{-1}$  ( $n = 5$ ) to  $4.15 \pm 1.16 \text{ nA mM}^{-1}$  (10 min,  $n = 3$ ,  $P = 0.0052$ ).

## Temperature treatment: number of bakings

Various temperature treatments of cast Nafion® films have been reported in the literature as a means of improving the properties of the film. Two of the most common include thermal annealing<sup>29</sup> and the high boiling point, substituted solvent method.<sup>30,31</sup> The former is the simpler of the two and has been used to protect metal-oxide pH electrodes, and to form anti-corrosion barrier layers.<sup>32,33</sup> The high boiling point, substituted solvent method involves substitution of the ethanol–water solvent, used to prepare the commercial Nafion® solution, with a high boiling point solvent (*e.g.* dimethylformamide or dimethyl sulfoxide). It has been shown with this method that the degree of crystallinity in the cast film increases with temperature, with the residual high-boiling point solvent argued to act as a plasticiser.<sup>31</sup>

The temperature range used in the treatments just described extends from 80 °C to 170 °C. We began our investigation of temperature by exposing both the six- and ten-coated electrodes to a temperature at the low end of this range, *i.e.* 85 °C. The results are summarised in Table 1.



**Fig. 1** Amperometric calibration plots for ascorbic acid (AA) measured in PBS, pH 7.4, at +0.7 V (*versus* SCE) at Pt-disk electrodes modified with Nafion® (5%) by dip coating and allowing a 2 min dry time between successive coats: 1 coat (●), 2 coats (×), 5 coats (◆), 6 coats (□) and 10 coats (▲). Inset: Close-up of calibration data for electrodes modified with 6 and 10 coats.

**Table 1** Effect of 85 °C temperature treatment on the 1 mM ascorbic acid sensitivity of Nafion®-modified Pt-disk electrodes<sup>a</sup>

Number of Nafion® coats	Baking time/min	One bake	Dip-bake
6	5	2.76 ± 1.09 (4)	—
10	4	3.08 ± 0.97 (7)	—
10	5	1.13 ± 0.45 (4)	0.93 ± 0.25 (11)
10	10	0.80 ± 0.30 (7)	—

<sup>a</sup> Number of electrodes in parentheses.

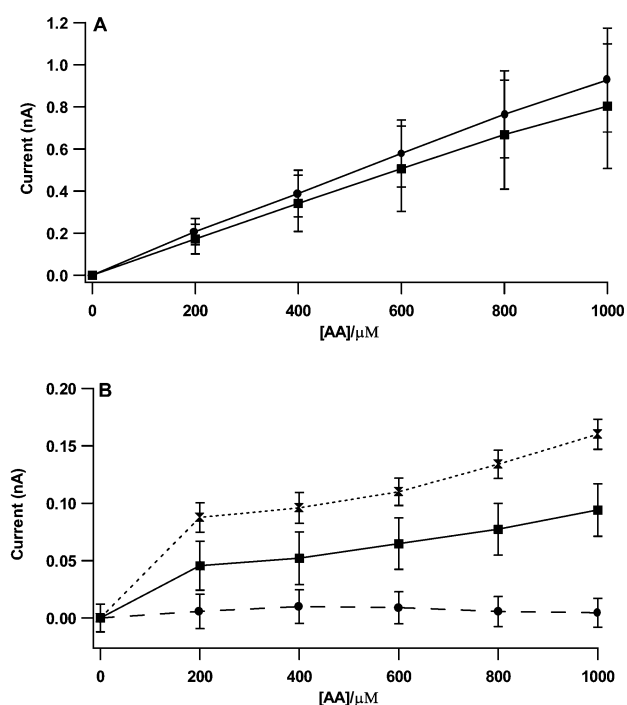
Annealing both electrode types for 5 min resulted in a deterioration in the blocking effect with the AA response increasing to  $2.76 \pm 1.09 \text{ nA mM}^{-1}$  ( $n = 4$ , 6-coats) and  $1.13 \pm 0.45 \text{ nA mM}^{-1}$  ( $n = 4$ , 10-coats). Increasing the annealing time from 5 min to 10 min for the 10-coated electrodes resulted in no significant increase in AA blocking ( $0.8 \pm 0.30 \text{ nA mM}^{-1}$ ,  $n = 4$ ,  $P = 0.5645$ ), while reducing it to 4 min produced a decrease to  $3.08 \pm 0.97 \text{ nA mM}^{-1}$  ( $n = 7$ ).

We next examined the effect of modifying the casting and annealing process by applying a dip-baking procedure which involved baking the electrode for 5 min between each coating. Application of 10 coats by this method produced no significant increase in blocking ( $0.93 \pm 0.25 \text{ nA mM}^{-1}$ ,  $n = 11$ ,  $P = 0.6899$ ) over the simpler single bake method of ten coats with five min baking (see Fig. 2A). Clearly, all the above modifications at 85 °C produced no improvement in the AA blocking ability over the 2 min dry no bake modification ( $0.52 \pm 0.06 \text{ nA mM}^{-1}$ ).

## Temperature treatment: baking temperature

We next examined the effect of annealing the electrodes at a temperature at the high end of the temperature range, *i.e.* 210 °C. Both the single bake and the dip-bake methods were investigated for different numbers of Nafion® coats. The results of these experiments are summarised in Table 2.

For the single bake 10 coats there was a decrease in AA sensitivity to  $0.26 \pm 0.13 \text{ nA mM}^{-1}$ ,  $n = 4$ . This is an improvement on our previous best blocking effect of  $0.52 \pm 0.06 \text{ nA mM}^{-1}$  (2 min dry no bake modification). Decreasing the number of coats to 5 resulted in a further improvement in AA blocking;  $0.09 \pm 0.04 \text{ nA mM}^{-1}$ ,  $n = 3$ ,  $P = 0.0033$ . Further reduction in the number of coats produced a deterioration in the blocking ability with decreased number of coats:  $0.23 \pm 0.16 \text{ nA mM}^{-1}$  (3 coats,  $n = 3$ ,  $P = 0.0922$ );  $1.29 \pm 0.68 \text{ nA mM}^{-1}$  (2



**Fig. 2** (A) Amperometric calibration plots for ascorbic acid (AA) measured in PBS, pH 7.4, at +0.7 V (*versus* SCE) at Pt-disk electrodes modified with Nafion® annealed at 85 °C for 5 min: ten coats single bake (●), ten coats dip-bake (■). (B) Amperometric calibration plots for ascorbic acid at Pt-disk electrodes modified with Nafion® annealed at 210 °C for 5 min using the dip-bake method: ten coats (●), five coats (×), three coats (■).

coats,  $n = 3$ ,  $P = 0.1748$ );  $6.70 \pm 4.31 \text{ nA mM}^{-1}$  (1 coat,  $n = 4$ ,  $P = 0.0317$ ).

Application of the dip-bake method produced a trend of increased sensitivity with decreased number of coats from 10 to 3: 10 ( $0.005 \pm 0.008 \text{ nA mM}^{-1}$ ,  $n = 3$ ), 5 ( $0.094 \pm 0.082 \text{ nA mM}^{-1}$ ,  $n = 4$ ) and 3 ( $0.160 \pm 0.116 \text{ nA mM}^{-1}$ ,  $n = 4$ ) coats (see Fig. 2B). Decreasing the number of coats to 2 produced no significant change in sensitivity from that observed with 3 coats ( $0.115 \pm 0.041 \text{ nA mM}^{-1}$ ,  $n = 3$ ,  $P = 0.7634$ ). Clearly, the dip-bake method produces the best AA blocking effect, with 10 coats being the optimum choice.

Dip-coating time

This was tested using the dip-bake (210 °C) method. 3 coats were applied using a dipping procedure which involved a direct dip in and out of the Nafion® solution. This resulted in an AA sensitivity of  $0.16 \pm 0.12 \text{ nA mM}^{-1}$ ,  $n = 3$ . Increasing the dipping time to 30 s produced no significant change in sensitivity ( $0.20 \pm 0.09$ ,  $n = 4$ ,  $P = 0.8093$ ) indicating that simply dipping the electrodes in and out of the Nafion® solution without a delay is sufficient to produce good blocking ability.

Concentrated pre-coats of Nafion® before baking

In an attempt to further improve the permselective characteristics of the cast Nafion® films to AA we developed a novel pre-coating method. Details are given in the Experimental section.

Following on from the previous section only the dip-bake method was investigated for 5 and 10 coats. The results of these experiments are summarised in Table 3. In some cases negative sensitivities are reported. In such cases, after a baseline measurement was taken, there was no response when AA was added, and the background current actually drifted slightly below the previously recorded baseline value.

Table 2 Effect of 210 °C temperature treatment on the 1 mM ascorbic acid sensitivity of Nafion®-modified Pt-disk electrodes<sup>a</sup>

Number of Nafion® coats	One bake	Dip-bake
10	$0.26 \pm 0.13$ (4)	$0.005 \pm 0.008$ (3)
5	$0.093 \pm 0.044$ (3)	$0.094 \pm 0.082$ (4)
3	$0.23 \pm 0.16$ (3)	$0.160 \pm 0.116$ (3)
2	$1.29 \pm 0.679$ (3)	$0.115 \pm 0.041$ (3)
1	$6.70 \pm 4.31$ (4)	—

<sup>a</sup> Number of electrodes in parentheses.

Table 3 Effect of 210 °C temperature treatment on the 1 mM ascorbic acid sensitivity of Nafion®-modified Pt-disk electrodes initially treated with concentrated pre-coats of Nafion®

Number of Nafion® coats	Number of Nafion® pre-coats <sup>b</sup>	
	10	5
10	—	$0.008 \pm 0.061$ (21)
5	—	$0.002 \pm 0.006$ (3)
3	$0.012 \pm 0.009$ (3)	$-0.005 \pm 0.006$ (6)
2	$-0.031 \pm 0.132$ (3)	$-0.033 \pm 0.017$ (6)
1	$0.027 \pm 0.050$ (3)	$0.115 \pm 0.107$ (3)

<sup>a</sup> Number of electrodes in parentheses. <sup>b</sup> Negative sensitivities are reported in cases where there was no response to ascorbic acid additions and the background current drifted slightly below the previously recorded baseline value over the time scale of the calibration (see Concentrated pre-coats of Nafion® before baking and Fig. 3).

For both the 10 and 5 pre-coats the 1 coat produced the poorest AA blocking effect;  $0.027 \pm 0.050 \text{ nA mM}^{-1}$ ,  $n = 3$  and  $0.115 \pm 0.098 \text{ nA mM}^{-1}$ ,  $n = 3$  respectively. Increasing the number of coats for both electrode types resulted in complete elimination of the AA response. As such, the final modification chosen against interference was 5 pre-coats and 2 dip coats, dip-baked at 210 °C. Typical calibration data is shown in Fig. 3. All data presented in the subsequent sections are for this type of electrode.

Uric acid interference

In addition to ascorbic acid the purine metabolite uric acid (UA) is another important potential interferent in sensors designed for *in vivo* measurements in the brain.<sup>34</sup> Its ECF concentration has been reported to be dependent on the size of the implanted sensor, with an approximate concentration of 50 μM determined using 300 μm diameter carbon paste electrodes.<sup>35</sup> UA calibrations were thus performed in the range 0–60 μM at both bare Pt and Nafion®-modified Pt electrodes. The sensitivity for 60 μM UA at bare Pt was  $1.84 \pm 0.03 \text{ nA}$  ( $n = 4$ ). Injection of 60 μM UA produced no change in the response of the Nafion®-modified (5 pre-coats, 2 dip coats) Pt electrodes:  $0.058 \pm 0.003 \text{ nA}$ ,  $n = 3$  (background);  $0.051 \pm 0.002 \text{ nA}$ ,  $n = 3$ ,  $P = 0.1347$

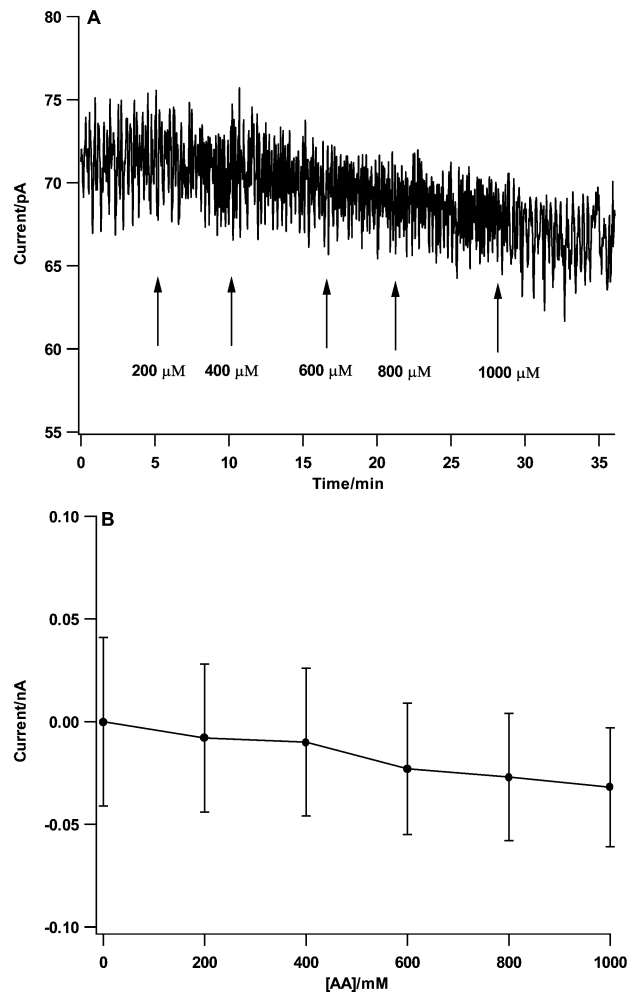


Fig. 3 (A) Typical current–time responses at Nafion®-modified Pt-disk electrodes in PBS, pH 7.4, at +0.7 V (versus SCE) for ascorbic acid (AA) in the concentration range 200–1000 μM. Modification with Nafion® involved applying an initial concentrated layer of 5 pre-coats and then 2 subsequent dip coats, dip-baked at 210 °C. Arrows indicate the points of injection. (B) Steady-state current concentration profile for AA calibration data obtained from electrodes ( $n = 3$ ) described in A.

(60  $\mu\text{M}$ ), indicating complete elimination of UA interference ( $P < 0.0001$  vs. bare Pt response).

### Dopamine detection

As outlined in the introduction Nafion<sup>®</sup>-modified electrodes have previously been used for the detection of electroactive cationic neurotransmitter species such as dopamine (DA) in the ECF.<sup>23,24</sup> DA calibrations were thus performed in the range 0–100  $\mu\text{M}$  at both bare Pt and Nafion<sup>®</sup>-modified Pt electrodes. The sensitivity for 100  $\mu\text{M}$  DA at bare Pt was  $7.25 \pm 0.47$  nA ( $n = 3$ ). 100  $\mu\text{M}$  DA produced no change in the response of the Nafion<sup>®</sup>-modified Pt electrodes:  $0.28 \pm 0.15$  nA,  $n = 3$  (background);  $0.26 \pm 0.14$  nA,  $n = 3$ ,  $P = 0.6804$  (100  $\mu\text{M}$ ), indicating complete blocking of the DA signal ( $P = 0.0001$  vs. bare Pt response). This is not too surprising as DA detection involving Nafion<sup>®</sup>-modified electrodes normally involves carbon fibre electrodes modified with the commercial 5% solution without temperature annealing. Other researchers have also observed a blocking of the dopamine signal with temperature treated Nafion<sup>®</sup>-modified electrodes.<sup>36</sup>

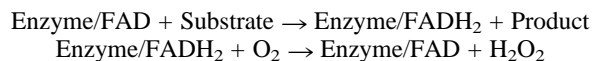
### Shelf life

After 14 days of storage at room temperature the AA sensitivity of the electrodes increased from  $0.007 \pm 0.002$  nA ( $n = 2$ ) to  $0.017 \pm 0.009$  nA ( $n = 2$ ). Although this represents an increase in the sensitivity over a 14 day period it is not significant ( $P = 0.4011$ ) and is still negligible and significantly less than for bare Pt ( $45.22 \pm 1.57$  nA  $\text{mM}^{-1}$ ,  $n = 30$ ,  $P < 0.0001$ ).

### Biosensor applications

Prolific research on the development and applications of biosensors, in the form of enzyme-modified electrodes, over the last three decades has yielded sensors for a large variety of substrates.<sup>37</sup> However, despite the significant advantage of continuous real time monitoring afforded by these electrochemical devices, only a small percentage have actually been used *in vivo* for chemical analysis of the living brain. The sensors that have been successfully used have all been amperometric flavoprotein oxidase-modified electrodes for either glucose, lactate, glutamate or choline.<sup>38</sup> A major factor limiting the application of biosensors in complex biological media is interference.

Most oxidase enzymes use molecular  $\text{O}_2$  as a mediator to produce the signal generating  $\text{H}_2\text{O}_2$ :



where FAD and  $\text{FADH}_2$  are the oxidized and reduced states of the redox active prosthetic group, flavin adenine dinucleotide. Thus, in order to investigate the suitability of our interference free Nafion<sup>®</sup>-modified electrodes for *in vivo* biosensor applications we examined the permeability of the membrane to  $\text{O}_2$  and  $\text{H}_2\text{O}_2$ .

Oxygen calibrations were performed in  $\text{N}_2$  and air at both bare Pt and Nafion<sup>®</sup>-modified Pt electrodes. No significant difference was observed in the sensitivities of both electrode types at 200  $\mu\text{M}$   $\text{O}_2$  (bare Pt:  $328 \pm 15$  nA,  $n = 4$ ; Nafion<sup>®</sup>-modified:  $231 \pm 36.43$  nA,  $n = 3$ ,  $P = 0.1333$ ) indicating that the Nafion<sup>®</sup> membrane is permeable to molecular  $\text{O}_2$ .  $\text{H}_2\text{O}_2$ , on the other hand, did show reduced sensitivity; the response at bare Pt was  $42.7 \pm 6.7$  nA ( $n = 3$ ), while that at the Nafion<sup>®</sup>-modified electrodes was  $11.5 \pm 2.5$  nA ( $n = 3$ ,  $P = 0.0121$ ). The detection limits, defined as the analyte concentration yielding a signal equal to three times the standard deviation of

the background current, for  $\text{O}_2$  and  $\text{H}_2\text{O}_2$  were 2.7  $\mu\text{M}$  and 34  $\mu\text{M}$  respectively.

### Conclusions

Elimination of interference from ascorbic acid and uric acid at a microelectrochemical Pt sensor targeted for *in vivo* applications was achieved using a novel procedure for coating Nafion<sup>®</sup> onto the electrode surface. The Nafion<sup>®</sup> was applied using a thermally annealing procedure involving 5 pre-coats, and 2 subsequent dip-bake layers. The optimum time and temperature for annealing was found to be 5 min at 210  $^\circ\text{C}$ . This procedure also produced complete blocking of the signal for the neurotransmitter dopamine. An examination of shelf life over two weeks indicated negligible AA interference over this period.

Although the modified electrode had no effect on  $\text{O}_2$  permeability it did produce a significant decrease in  $\text{H}_2\text{O}_2$  sensitivity. This may preclude use of this Nafion<sup>®</sup> coating methodology in the design of implantable, first generation, peroxide detecting biosensors. However, since there was no blocking effect for molecular oxygen this type of Nafion-modified electrode may be more suitable for detection of gaseous neurochemicals such as nitric oxide. Future work will focus on investigating this, and potential biosensor use using the model glucose oxidase as the immobilized enzyme.

### Acknowledgement

We gratefully acknowledge financial support from Enterprise Ireland (FOB, Ph.D Studentship, 1999–2002 – BR/1999/159), the Health Research Board (EQN12/2000), and the National University of Ireland, Maynooth (New Researcher Award) for financial support.

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